

REMARKS

Status of the Claims

Applicants appreciate the Examiner's careful review of the independent and dependent claims in the outstanding Office Action. Confirming a restriction requirement made on the telephone, the Examiner stated that the claims of this invention were restricted to one of the following inventions:

- I. Claims 1-27, drawn to a hyperbranched dendron, classified in class 525, subclass 92B, 178, 179.
- II. Claims 28-77, drawn to a complex between a hyperbranched dendron polymer and a nucleic acid molecule, classified in class 424, subclass 78.08+.

Applicants hereby confirm their provisional election of Group I, and claims 28 to 77 are cancelled herein without prejudice as to their presentation in a divisional, continuation, or other application.

Accordingly, claims 1-27 were examined in the outstanding Office Action, with claims 1, 11 and 20 being independent claims. All of these claims (1-27) stand rejected.

In this amendment, claim 1 has been amended to move language from the preamble to the body of the claim. This is not a narrowing amendment as the language is already used in claim 1 to define the present invention, the language is merely being reformatted. In addition, new dependent claims 78 to 82 have been added. Claims 78, 80 and 82 recite that the claimed polymer is polydisperse. This amendment is supported throughout the application, including at page 9, line 18 to page 10, line 9. Claims 79 and 81 depend from claims 11 and 20 respectively and recite that the polymer is a flexible hyperbranched dendron as is recited in claim 1. Claims 10, 19 and 21 have been amended to correct typographical errors. Accordingly, these amendments and additions add no new matter.

Overview Of The Present Invention

The present invention relates to the synthesis and use of polycationic polymers to mediate the non-viral transfection of genetic material into cells, both *in vitro* and *in vivo*. The

invention involves the synthesis of flexible hyperbranched dendron polymers using a branched polyethyleneimine (B-PEI) core. The polymer is synthesized via iterative addition of 2-chloroethyl amine to B-PEI under basic conditions to form polymers with primary amine groups at the surface, while the secondary and tertiary amine groups are in the interior and at the core of the polymer. This synthetic strategy allows the preparation of randomly branched polymers, with molecular weight of 10 to 25KD, and a secondary to tertiary amine ratio less than or equal to 1.5 to 1.0.

The hyperbranched dendron (HD) polymers are polydisperse and their extensive branching and flexible architecture allows these polymers to complex nucleic acids tightly, to give nanoparticles having a diameter of 50 to 100 nm. The smaller diameter of the HD-nucleic acid conjugate improves the ability of these polymers to transfect genetic material into cells even in the presence of the anionic biopolymer serum. The transfection efficiency is a function of the ratio of positive charge (from the polymer) to negative charge (from nucleic acid) in the HD-nucleic acid conjugate (N/P ratio). The synthesis of HD via consecutive aminoethylation of surface groups, allows preparation of polymers having different surface charges and different cores. Therefore, the neutralization point at which the polymer will release its cargo in the interior of the cell can be altered. The lower N/P ratios (N/P = 5-10) of HD polymers, versus hyperbranched comb polymers (N/P = 200 – 1000) suggest that a much lower amount of polymer is required to effectively transfect genetic material, and hence a low overall toxicity to cells during transfection.

The HD polymers exhibit lower cell toxicity than the commercially available low molecular weight PEI (10KD), and high molecular weight PEI (~25KD) polymers. The lower cellular toxicity of HD is presumably due to a higher secondary to tertiary amine content of the HD polymer. Furthermore, the lower toxicity and high stability of the DNA-polymer conjugates of the present invention allows for the prolonged expression of the desired gene both in-vitro and in-vivo.

Finally, the HD polymers form very stable complexes with plasmid vectors. These conjugates can be stored as lyophilized powders which are reconstituted into solution prior to use. The high stability also allows for systemic and targeted delivery of genetic material. Thus

the advantages of HD polymers over other branched PEI polymers are: (1) ease of synthesis, resulting in a random flexible polymer that has a high transfection efficiency at lower N/P ratios; (2) polymer forms stable complexes with DNA; (3) tolerance to serum; (4) low toxicity of HD-DNA conjugates as the amount of HD required for efficient DNA delivery into cells is low.

Rejection of Claims 1-27 Under 35 U.S.C. 102(b)

The Examiner states that claims 1-27 are rejected under 35 U.S.C. 102(b) as being anticipated by Yin et al. (U.S. Patent No. 5,919,442).

Specifically, the Examiner states:

Yin discloses a hyper-branched polymer comprising a core of polyethyleneimine and reactive sites of said core having functional amine groups, see Fig 9 and Fig. 28. Reference discloses a linear polyethyleneimine core having surface modification performed by grafting a plurality of amine groups. The maximum diameter of a hyper comb-branched polymer is about 100 nm, col. 5, line 27. Molecular weights are in the range of 10,000 to about 100,000,000, col. 7, line 35. The hyper comb-branched PEI having primary amine modifications can be functionalized with secondary amines for producing -NH₂ terminal groups. The resulting polymer can have multilayered dendritic architecture having more than one hydrophilic layer, col. 5, lines 1-67 and col. 6, lines 1-67. Chloroethylamine modified hyper comb-branched polymer is readable in applicants' claimed hyperbranched dendron polymer, col. 21, lines 28, 38 and 51; col. 30, lines 36-37; col. 32, lines 5-8 and 13 for the present claims 10, 19, and 21. The elongated=linear core having a plurality of arms branched therefrom is grafted to produce a grafting layer having amine terminal groups, col. 13, lines 59-61 and col. 24, lines 24-26. Yin discloses a terminal primary amine modified hyper comb-branched polymers and a secondary amine modified hyper comb-branched polymer, col. 32, lines 8- and 13. The tertiary amine groups grafted on the core are inherent in Yin invention because the functionality of amine groups is depending on the desired surface modification property and since reference discloses multilayered dendrimer.

Applicants disagree.

Overview of Yin Patent (U.S. Patent No. 5,919,442)

Yin describes the synthesis and use of a hyperbranched comb polymer dendron consisting of a linear polyethyleneimine core having multiple reactive sites (Fig. 1). Generational branches are sequentially added onto the core (Fig 2), each branch is a living polymer that is synthesized separately and has one reactive end group and multiple reactive sites along the backbone of the living polymer. The reactive sites of the living polymer are protected during synthesis and should not be able to react intramolecularly. Polymer is synthesized by adding living polymers to the reactive sites of either the core, or reactive sites of branches of a previous generation (Col. 6, lines 1-67). By varying the grafting densities, space between reactive sites on the core and early generation branches, polymers with different interior void volumes can be synthesized. The synthetic protocol also allows for the rapid generation of high molecular weight polymers, which have a high degree of order, a diameter of about 100nm and molecular weights in the range of 10,000 to 100,000,000.

Hyperbranched comb polymers are monodisperse, and in one embodiment of the patent Yin describes the use of 2-ethyl-1,3oxazoline as a synthon to generate both the core, as well as the living polymer that is grafted onto the core (Col. 11, lines 64-67, Col. 12, lines 1-67). The branches of the final generation can be selectively modified to include desired functionality (NH₂, COOH etc.), which will allow the polymer to complex and transport various agents such as nucleic acids, diagnostic agents, agricultural agents, bioactive, and industrial agents into cells. Although Yin describes the use of 2-chloroethylamine to selectively modify the surface amine groups of the polymer (Col. 21, line 38), there is no mention of using 2-chloroethylamine as a monomer to synthesize the hyperbranched comb dendron. The invention of Banerjee et. al., specifically uses 2-chloroethylamine during polymer synthesis.

Yin describes the use of hyperbranched comb dendron polymer as a carrier to transport genetic material into cells. Transfection is generally carried out using a polymer : DNA charge ratio (N/P) of 100 to 10,000, and preferably at a ratio of 200 (col. 29, lines 12-18). Yin also describes the transfection efficiency of the hyperbranched comb dendron polymers to other

genetic material carriers such as Lipofectamine and Starburst polymers. The hyperbranched comb polymers whose surfaces were modified as primary amines were more efficient at transporting DNA into cells versus Lipofectamine and Starburst polymers. The N/P ratios for efficient transfection were in the range of 100 to 500 (Fig. 29 and 30, Col. 31, lines 18-22).

Hyperbranched comb polymers have lower toxicity than randomly branched PEI polymers even at N/P ratio equal to 100. However, the transfection efficiency is maximal at a N/P ratio of 200. This N/P ratio is significantly higher than that required for transfection using the hyperbranched dendron polymer of Banerjee, requiring the use of greater quantities of the comb polymer for efficient transfection, which could translate into higher toxicity for the comb polymer-DNA conjugates versus the hyperbranched dendron polymer-DNA conjugates.

Response to Rejection

Claim 1 recites a *flexible hyperbranched dendron polymer* having a *branched polyethyleneimine core*, a plurality of primary amine groups on the polymer surface and a plurality of secondary and tertiary amine groups positioned in the core. In contrast, Yin teaches of a *hyper comb-branched polymer* wherein the *core is a linear polymer of ethyleneimine moieties*. The use of a branched polyethyleneimine core in claim 1 allows the synthesis of a randomly branched structure, of greater flexibility (flexibility is also recited in claim 1) than the hyper comb-branched polymer of Yin. This flexibility allows hyperbranched dendron polymer to effectively complex nucleic acids, and form compact nanoparticles. Because Yin does not teach a branched core as recited – in fact, *the Examiner describes Yin in the Office Action as disclosing “a linear polyethyleneimine core”* – it cannot anticipate claim 1. Yin further fails to teach that the resulting polymer is flexible (because Yin’s is not).

Further, Yin provides no disclosure, teaching or suggestion of a plurality of *primary amine groups on the polymer surface* and a plurality of *secondary and tertiary amine groups positioned in the core*. The specification of the present patent application discloses synthesis of the hyperbranched dendron polymer by iterative addition of ethyleneimine monomers to the polyethyleneimine core. Yin illustrates the synthesis of a hyper comb-branched dendrimer via iterative addition of “living polymers” to the reactive sites of either the polyethyleneimine core or dendrons from a previous generation. There is no mention in Yin of synthesizing the comb-

branched polymer via the addition of monomeric polyethyleneimine units to the reactive sites of the core or dendrons from a previous generation. The ability to synthesize polymers via the iterative addition of monomeric units has many advantages, such as ease of synthesis, the ability to increase the ratio of branched to linear structures in the polymer, and the ability to increase the secondary and tertiary amine content of the polymer while maintaining the plurality of primary amine groups on the surface of the polymer.

Yin does not disclose, teach or suggest providing a plurality of primary amine groups on the polymer surface and a plurality of secondary and tertiary amine groups positioned in the core. Nor does Yin disclose, teach or suggest a synthesis method by which such a structure can be made. For this additional reason, Yin does not anticipate claim 1.

Because polymers of the invention can be synthesized in a manner that allows precise control over the stoichiometries of primary, secondary and tertiary amine groups in the polymer molecule, functionally, these polymers have an advantage over hyper comb-branched and other dendritic polymers due to their high buffering capacity, ability to form compact nanoconjugates with nucleic acid and the increased stability of these nanoconjugates in solution.

Yin also fails to anticipate independent claim 11 which recites a *flexible hyperbranched dendron polymer* having a *randomly branched structure* and a *ratio of secondary to tertiary amine groups of less than or equal to about 1.5 to 1*. The polymers of Yin are not randomly branched – but are comb-branched based on linear core. Further Yin provides no disclosure, teaching or suggestion as to the flexible hyperbranched nature of the claimed polymer. Further, the Examiner points to no disclosure, and Applicants can find none in Yin, that relates to the ratio of secondary to tertiary amine groups. This ratio, especially when combined with the recited molecular weight ratio of 10 to 25 kD (it is known that that highly branched, commercially available PEI have high molar mass ranging from 25K to 800K) suggests that successive aminoethylation resulted in an increase of branching (Table 1 of the application) and that the increased branching enables the process to incorporate more secondary and tertiary amines than present in commercially available PEI core. Without limiting the invention to one advantage, this resulted in an observed concomitant reduction of cytotoxicity of hyperbranched dendrons compared to a commercial PEI core (See Fig 2 of the application and accompanying

text). These features and their advantages are not disclosed taught or suggested in Yin, and accordingly, claim 11 is not anticipated by Yin.

Independent claim 20 recites a *hyperbranched dendron polymer* made by a process that *increases the amount of secondary and tertiary amines in the polymer while maintaining a plurality of primary amines on a surface of the polymer*. As explained above, the ability to branch polymers via the iterative addition of monomeric units as taught in the present patent application has many advantages, including the ability to increase the secondary and tertiary amine content of the polymer while maintaining the plurality of primary amine groups on the surface of the polymer. Functionally these polymers have an advantage over hyper comb-branched and other dendritic polymers due to their high buffering capacity, ability to form compact nanoconjugates with nucleic acid and the increased stability of these nanoconjugates in solution. As Yin provides no disclosure, teaching or suggestion of this claimed structure (nor of the method of achieving it), it cannot anticipate claim 20.

At one point in the rejection, the Examiner notes that “the tertiary amine groups grafted on the core are inherent in Yin invention because the functionality of amine groups is depending on the desired surface modification property and since reference discloses multilayered dendrimer.” As the Examiner implicitly notes, Yin does not disclose maintaining primary amine groups at the surface of the polymer while increasing secondary and tertiary amine content at the core. That polymers can be tailored to have specific surface properties is not a substitute for an anticipatory disclosure when Yin (1) provides no disclosure of Applicants’ claimed structure, (2) provides no disclosure of a method that will result in Applicants’ claimed structure, and (3) provides no disclosure of the advantages achieved by achieving Applicants’ claimed structure. Finally, “The doctrine of *inherency* is available *only* when the prior inherent event can be established as a *certainty*. That an event *may result* from a given set of circumstances is *not sufficient* to establish anticipation. Probabilities are not sufficient. ... A prior inherent event cannot be established based upon speculation or where a doubt exists.” *Ethyl Molded Products Co. v. Betts Package Inc.*, 9 USPQ2d 1001, 1032-33 (E.D. Ky. 1988). “*To establish inherency*, the extrinsic evidence ‘must make clear that the missing descriptive matter is *necessarily present* in the thing described in the reference, and that *it would be so recognized by persons of ordinary skill*.’ ... ‘Inherency, however, may not be established by probabilities or possibilities.


The mere fact that a certain thing may result from a given set of circumstances is not sufficient.” In re Robertson, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999) In addition, “[i]n relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic *necessarily* flows from the teachings of the applied prior art.” *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Int’f 1990). These high standards for anticipation by inherency are not met in this case.

CONCLUSION AND REQUEST FOR TELEPHONE INTERVIEW

In view of the above amendment, Applicants believe the pending application is in condition for allowance. Applicants believe that prosecution of the present application could benefit from a telephone interview between Applicants' representatives and the Examiner. Accordingly, the undersigned representative will contact the Examiner in an effort to identify a mutually agreeable time for an interview.

Dated: January 9, 2006

Respectfully submitted,

By 

Ronald E. Cahill

Registration No. 38,403

NUTTER MCCLENNEN & FISH LLP

World Trade Center West

155 Seaport Boulevard

Boston, Massachusetts 02210-2604